

# The Yellow Sea green tides were dominated by one species, *Ulva* (*Enteromorpha*) *prolifera*, from 2007 to 2011

ZHAO Jin<sup>1,2</sup>, JIANG Peng<sup>1\*</sup>, LIU ZhengYi<sup>3</sup>, WEI Wei<sup>4</sup>, LIN HanZhi<sup>1,2</sup>, LI FuChao<sup>1</sup>, WANG JinFeng<sup>1</sup> & QIN Song<sup>1\*</sup>

<sup>1</sup> Key Laboratory of Experimental Marine Biology, Institute of Oceanology, Chinese Academy of Sciences, Qingdao 266071, China;

<sup>2</sup> Graduate University of Chinese Academy of Sciences, Beijing 100049, China;

<sup>3</sup> College of Resources and Environmental Sciences, Nanjing Agricultural University, Nanjing 210095, China;

<sup>4</sup> Department of Biology, Science College, Shantou University, Shantou 515063, China

Received May 4, 2012; accepted July 11, 2012; published online August 16, 2012

Green tides have occurred in the Yellow Sea successively from 2007 to 2011. Genetic analysis of the 5-year green-tide-forming algae needs to be performed to determine the source of the biomass and understand the mechanism of the green tide blooms. In this study, free-floating green algae were collected at different sites in the Yellow Sea in 2010 and 2011. Data on 182 free-floating samples and 155 attached *Ulva* samples from previous studies on the Yellow Sea green tides from 2007 to 2009 were also taken into consideration. Morphology observation and molecular phylogenetic analyses indicated that the Yellow Sea green tides were dominated by a single species, *Ulva prolifera*, from 2007 to 2011. Considering that at least five *Ulva* species inhabit the north coast of China, the unialgal composition of the green tides implied that (1) there may be some special physiology and propagation pathways of *U. prolifera* for its rapid expansion, (2) the mechanisms of the Yellow Sea green tide formation were similar for the last five years, and (3) the intra-species genetic variation and population structure of *U. prolifera* need to be studied to determine the exact origin of the bloom-forming biomass.

**green tide, species identification, the Yellow Sea, *Ulva prolifera***

**Citation:** Zhao J, Jiang P, Liu Z Y, et al. The Yellow Sea green tides were dominated by one species, *Ulva* (*Enteromorpha*) *prolifera*, from 2007 to 2011. Chin Sci Bull, 2013, 58: 2298–2302, doi: 10.1007/s11434-012-5441-3

Macroalgal blooms are serious environmental problems in coastal ecosystems around the world [1–3]. In China, masses of free-floating green algae have accumulated in the Yellow Sea successively in summer from 2007 to 2011. In contrast to most algal blooms that occur in eutrophicated closed or semi-closed bays [4,5], the Yellow Sea green tides occurred in an open sea area on a much larger scale, and the bloom-forming biomass underwent long-distance transference [6]. Therefore, the exact species composition of the green tides must be determined to identify the biomass origin.

*Ulva*, including the former genus *Enteromorpha* [7], plays a principal role in most green tides around the world

[2,5]. Internal transcribed spacer (ITS) sequences are the most widely used molecular markers for species identification for this genus. Using ITS sequences, Jiang et al. [8] demonstrated that the free-floating *Ulva* in the Yellow Sea in 2007 were composed of one species belonging to the *Ulva linza-procera-prolifera* (LPP) clade, and the attached *Ulva* species from Qingdao coasts were not the biogeographic origin of the free-floating biomass in that season.

However, ITS cannot distinctly separate *U. linza* and *U. prolifera* [9], which are the common algae in the intertidal areas along the coast of China. To address this problem, morphological characteristics and 5S rDNA spacer sequences were used to confirm the species composition of the Yellow Sea green tides. The morphological presence (*U. prolifera*) or absence (*U. linza*) of branches was coincided

\*Corresponding authors (email: jiangpeng@qdio.ac.cn; sqin@qdio.ac.cn)

with the two phylogenetic discrete clusters based on 5S rDNA spacer [10]. Wang et al. [11] illustrated that the blooming algae in 2007 and 2008 were *U. prolifera* based on both morphological characteristics and ribotype network of ITS sequences. Liu et al. [12,13] proved that the dominant free-floating *Ulva* species in 2008 and 2009 possibly belonged to a single strain of the LPP clade, and the dominant bloom-forming alga was unlikely able to survive in the coastal waters in the winter conditions of Qingdao. Zhang et al. [14] demonstrated that the ITS and 5S spacer sequences of the algae covering the large area of the Yellow Sea in 2008 and 2009 exhibit high similarities, and the bloom-forming algae were identified as *U. prolifera*.

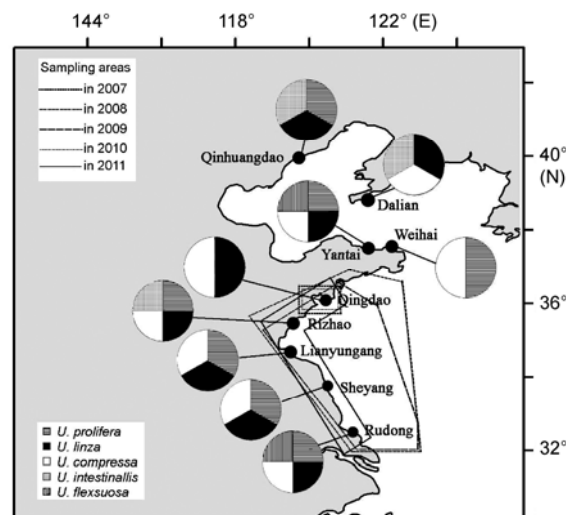
Although most previous studies indicated that one algal species formed the Yellow Sea green tides [15–17], there were some new discoveries in the recent two years. Based on morphological characteristics and molecular phylogenetic analyses, Duan et al. [18] distinguished three free-floating *Ulva* species, namely, *U. prolifera*, *U. compressa*, and *U. pertusa*; species diversity of the Yellow Sea free-floating *Ulva* appeared to be greater than previously thought. Tian et al. [19] pointed out that a succession of free-floating *Ulva* species, including *U. compressa*, *U. flexuosa*, and LPP complex, appeared in Rudong coastal areas, and the LPP strain was the same as the dominant *Ulva* species (*U. prolifera*) of the Yellow Sea green tides in 2008.

Species diversity of the *Ulva* genus can be observed along the coast of China (Figure 1), and the Yellow Sea green tides grew at different rates and had differences in spatial as well as temporal patterns in different years [30]. Determining whether species compositions of the green tides over the last five years were the same, and whether the green tides comprised one or several species are essential in identifying the source of the free-floating biomass and understanding the mechanism underlying the Yellow Sea green tide formation. In the present study, we reviewed previous studies on the species identification of the Yellow Sea green tides from 2007 to 2009, and complemented the free-floating samples in 2010 and 2011. The bloom-forming species of the Yellow Sea green tides and their genetic diversity over the last five years were discussed.

## 1 Materials and methods

Data on the Yellow Sea free-floating and attached *Ulva* (especially tubular *Ulva*) samples from previous studies were collected. A total of 182 free-floating samples collected from 2007 to 2009, and 155 attached *Ulva* samples distributed along the Chinese coast were considered (Figure 1 and Table 1).

In 2010 and 2011, free-floating green algae were collected from different sites along the coast of the Yellow Sea during the green-tide blooming season (Figure 1 and Table 1). Algal thalli were rinsed carefully in sterilized seawater



**Figure 1** Sampling areas and distribution of attached tubular *Ulva* spp. along the coast of the Yellow Sea and Bohai Sea. This map integrates information from many relevant studies on the Yellow Sea green tides and the attached *Ulva* species distribution along the north coast of China [8,11–15,18,20–29].

to remove debris and epiphytes. Morphological observation was conducted under a Nikon Eclipse 50i microscope (Nikon, Kawasaki, Japan).

Genome DNA was extracted by a modified CTAB method [31]. PCR amplification of the ITS region (including ITS1, 5.8S, and ITS2) was performed using the primers and thermal cycling profile of Leskinen et al. [32]. The PCR products were checked on 2% TAE agarose gels and sequenced by Sunny Bio Co. (Shanghai, China).

Sequences were aligned and edited using Clustal X [33]. Phylogenetic trees were built using the maximum parsimony (MP) and neighbor-joining (NJ) methods with Mega 4.0 [34] using *Blidingia minima* as the outgroup taxon. The robustness of the MP and NJ trees was tested by bootstrapping with 1000 replicates of the data.

## 2 Results and discussion

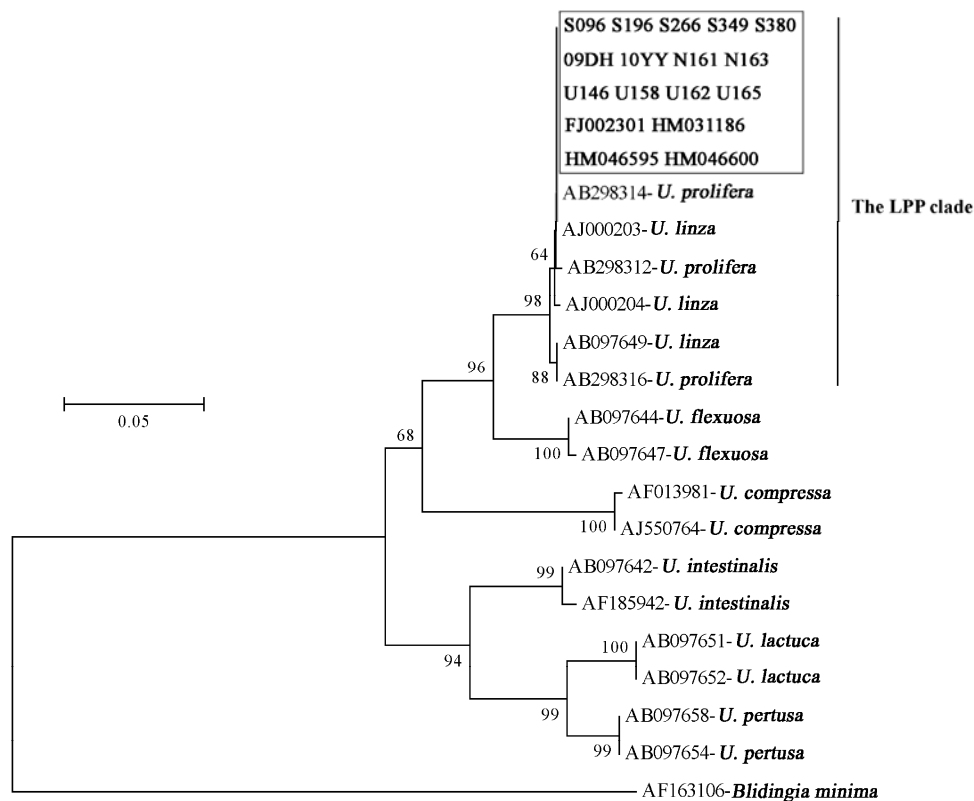
The Yellow Sea free-floating green algae sampled in 2010 and 2011 in this study had the same morphological characteristics as previous studies described about the dominant bloom-forming species from 2007 to 2009: highly branched, linear, tubular, and hollow thalli with monostromatic walls (as photos shown in refs. [11,15,16]). Their ITS sequences were identical and clustered in the same clade with the LPP group (Figure 2). Considering the morphological characteristics, the free-floating algae were identified as the same species, *U. prolifera*.

The ITS sequences of the free-floating samples in 2010 and 2011 collected in this study were identical with NCBI sequences FJ002301 and HM046600, which were from the dominant species of the Yellow Sea green tides in 2008 and

**Table 1** Details of *U. prolifera* samples and referent sequences used in this study<sup>a)</sup>

| References | Samples/number of samples        | Collection site or area   | Collection date        | Methods used for species identification |
|------------|----------------------------------|---|------------------------|---|
| This study | 10YY (F)                         | No.1 bathing beach, QD  | 23 Jul 2010            | ITS and M                               |
|            | U146 (F)                         | Zhujialin, Lvsj, Qidong, JS   | 2 Jul 2011             |   |
|            | U156 (F)                         | Haitou, Ganyu, JS   | 4 Jul 2011             |   |
|            | U158 (F)                         | Guanxi, JS  | 4 Jul 2011             |   |
|            | U165 (F)                         | Jiaonan, QD   | 5 Jul 2011             |   |
|            | N161 (F)                         | No.1 bathing beach, QD  | 7 Jul 2011             |   |
|            | N163 (F)                         | Haichuan Road, QD   | 10 Jul 2011            |   |
| [8]        | 10 (F); 15 (A)                   | 15 sites (QD), 119°45'–120°15'E, 35°30'–36°15'N   | Jul–Aug 2007           | ITS and M                               |
| [25]       | 4 (F)                            | 2 sites (QD)  | Jul 2008               | ITS and <i>rbcL</i>                     |
| [11]       | 21 (F)                           | 19 sites (YS), 119°32'–122°00'E, 32°25'–36°49'N   | Jun–Jul 2008           | ITS, <i>rbcL</i> , <i>psbA</i> and M    |
| [26]       | 70 (F)                           | 10 sites (YS) and 4 sites (QD), 120°00'–121°45'E, 33°44'–36°15'N  | the summer of 2008     | ITS and M                               |
| [15]       | 22 (F)                           | 20 sites (YS), 1 site (QD)  | Jul 2008               | ITS and M                               |
| [29]       | 7 (F)                            | QD, LYG and RD coastal areas  | Jun–Jul 2008           | ITS and 18S rDNA                        |
| [18]       | 3 (A); 3 (F, 2008); 16 (F, 2009) | 10 sites (QD), 2 sites (WH), 2 sites (YS), 1 site (YC), 120°12'–121°39'E, 35°57'–36°49'N  | Jul–Aug 2008; Jul 2009 | ITS, <i>rbcL</i> and 5S-NTS             |
| [12]       | 8 (F, 2008); 20 (F, 2009)        | 2 sites (QD, 2008), 25 sites (YS, May 2009, 120°00'–123°00'E, 32°00'–35°30'N); 18 sites (YS, June 2009, 120°00'–122°30'E, 33°30'–36°00'N) | Jun 2008; May–Jun 2009 | ITS, <i>rbcL</i> and M                  |
| [20]       | 18 (A)                           | 12 sites (Chinese coast), 119°33'–120°46'E, 34°05'–36°15'N  | Jun–Aug 2007           | ITS and <i>rbcL</i>                     |
| [14]       | 1 (F), 30 (A)                    | 11 sites (JS), 119°00'–121°30'E, 32°00'–35°00'N   | Mar–Apr 2009           | ITS and 5S-NTS                          |
| [27]       | 12 (A)                           | QD  | Apr 2009               | ITS and M                               |
| [28]       | 17 (A)                           | various habitates in JS, 119°28'–120°30'E, 33°49'–34°30'N   | May–Sep 2009           | ITS and M                               |
| [24]       | 60 (A)                           | 30 sites along Chinese coast  | 2009–2010              | ITS, 5S-NTS and M                       |
| Total      | 189 (F); 155 (A)                 |   | 2007–2011              |   |

a) A: attached; F: free-floating; JS: Jiangsu Province; LYG: Lianyungang City; M: morphology; QD: Qingdao City; RD: Rudong City; WH: Weihai City; YC: Yancheng City; YS: Yellow Sea.



**Figure 2** Phylogenetic tree of ITS sequences constructed by the neighbor-joining method. S096, S196, S266, S349, S380, and 09DH are the Yellow Sea free-floating samples collected from 2007 to 2009 [11,41]. AJ002301 represents 70 identical free-floating samples in 2008 [26]. HM031186, HM046595, and HM046600 represent 21 free-floating samples in 2009 [12,14].

2009, respectively. This result confirmed that the green tides in the Yellow Sea were dominated by a single species, *U. prolifera*, for the last five years. Although *U. compressa* and *U. pertusa* were also reported in some studies [12,18], the number of samples is really small (5 and 3, respectively) compared with the large number of *U. prolifera* samples (181 samples).

For the last five years, the Yellow Sea green tides were dominated by one species, *U. prolifera*. This finding is important to further studies. First, there are at least five tubular *Ulva* species along the north coast of China (Figure 1), but the green tides were unialgally composed. *U. prolifera* exhibits reproductive diversity and excellent ability in enduring adverse conditions, such as extreme hyper salinity and desiccation [35–37], which may help them drift a long distance on the sea surface. ESTs have been sequenced and annotated [38,39], but the functions of the annotated genes have not been validated and species-specific genes cannot be annotated. More studies on the physiology and proliferation of free-floating algae are needed. Second, the unialgal composition of the green tides implied the limited origin area of the free-floating biomass and similar mechanisms of the Yellow Sea green tide formation for the last five years. Lin et al. [40] pointed out that temporary cyclonic eddies in the Yellow Sea in late spring and early summer may help promote the proliferation of this bloom by providing seaweeds with sufficient growth time, abundant nutrition, and favorable habitats. Continuous research covering more comprehensive physical environment information is needed to understand the process of the green tide gestation, expansion, and declination. Third, preliminary studies on the intra-species genetic diversity suggested a close genetic relationship among the floating *U. prolifera* from 2007 to 2009 [41]. Given that *U. prolifera* is widely distributed along the Chinese coastal areas (Figure 1), more studies on the genetic structure and variance of the 5-year free-floating algae and attached populations of *U. prolifera* are necessary to better understand the source of the bloom-forming biomass and solve the severe environmental problem.

This work was supported by the Public Science and Technology Research Funds Projects of Ocean (201205010), Promotive Research Fund for Excellent Young and Middle-aged Scientists of Shandong Province (2010BSB02009), and the National Science & Technology Pillar Program of China (2008BAC49B01).

- 1 Fletcher R L. The occurrence of “green tides”: A review. In: Schramm W, Nienhuis P H, eds. Marine Benthic Vegetation: Recent Changes and the Effects of Eutrophication. Berlin: Springer, 1996. 7–43
- 2 Blomster J, Back S, Fewer D P, et al. Novel morphology in *Enteromorpha* (Ulvophyceae) forming green tides. *Am J Bot*, 2002, 89: 1756–1763
- 3 Yabe T, Ishii Y, Amano Y, et al. Green tide formed by free-floating *Ulva* spp. at Yatsu tidal flat. *Jpn Limnol*, 2009, 10: 239–245
- 4 Kostamo K, Blomster J, Korpelainen H, et al. New microsatellite markers for *Ulva intestinalis* (Chlorophyta) and the transferability of

- markers across species of Ulvaceae. *Phycologia*, 2008, 47: 580–587
- 5 Shimada S, Hiraoka M, Nabata S, et al. Molecular phylogenetic analyses of the Japanese *Ulva* and *Enteromorpha* (Ulvales, Ulvophyceae), with special reference to the free-floating *Ulva*. *Phycol Res*, 2003, 5: 99–108
- 6 Liu D Y, Keesing J K, Xing Q U, et al. World’s largest macroalgal bloom caused by expansion of seaweed aquaculture in China. *Mar Pollut Bull*, 2009, 58: 888–895
- 7 Hayden H S, Blomster J, Maggs C A, et al. Linnaeus was right all along: *Ulva* and *Enteromorpha* are not distinct genera. *Eur J Phycol*, 2003, 38: 277–294
- 8 Jiang P, Wang J F, Cui Y L, et al. Molecular phylogenetic analysis of attached Ulvaceae species and free-floating *Enteromorpha* from Qingdao coasts in 2007. *Chin J Oceanol Limnol*, 2008, 26: 276–279
- 9 Shimada S, Yokoyama N, Arai S, et al. Phylogeography of the genus *Ulva* (Ulvophyceae, Chlorophyta), with special reference to the Japanese freshwater and brackish taxa. *J Appl Phycol*, 2008, 20: 979–989
- 10 Hiraoka M, Ichihara K, Zhu W R, et al. Culture and hybridization experiments on an *Ulva* clade including the Qingdao strain blooming in the Yellow Sea. *PLoS One*, 2011, 6: e19371
- 11 Wang J F, Jiang P, Cui Y L, et al. Molecular analysis of green-tide-forming macroalgae in the Yellow Sea. *Aquat Bot*, 2010, 93: 25–31
- 12 Liu F, Pang S J, Xu N, et al. *Ulva* diversity in the Yellow Sea during the large-scale green algal blooms in 2008–2009. *Phycol Res*, 2010, 58: 270–279
- 13 Liu F, Pang S J, Chopin T, et al. The dominant *Ulva* strain of the 2008 green algal bloom in the Yellow Sea was not detected in the coastal waters of Qingdao in the following winter. *J Appl Phycol*, 2010, 22: 531–540
- 14 Zhang X W, Xu D, Mao Y Z, et al. Settlement of vegetative fragments of *Ulva prolifera* confirmed as an important seed source for succession of a large-scale green tide bloom. *Limnol Oceanogr*, 2011, 56: 233–242
- 15 Zhang X W, Mao Y Z, Zhuang Z M, et al. Morphological characteristics and molecular phylogenetic analysis of green tide *Enteromorpha* sp. occurred in the Yellow Sea (in Chinese). *J Fish Sci China*, 2008, 15: 822–829
- 16 Ding L P, Luan R X. The taxonomy, habit, and distribution of a green alga *Enteromorpha prolifera* (Ulvales, Chlorophyta) (in Chinese). *Chin J Oceanol Limnol*, 2009, 40: 68–71
- 17 Sun S, Wang F, Li C L, et al. Emerging challenges: Massive green algae blooms in the Yellow Sea. *Nat Proc*, 2008, hdl: 10101/npre.2008.2266.1
- 18 Duan W J, Guo L X, Sun D, et al. Morphological and molecular characterization of free-floating and attached green macroalgae *Ulva* spp. in the Yellow Sea of China. *J Appl Phycol*, 2012, 24: 97–108
- 19 Tian X L, Huo Y Z, Chen L P, et al. Molecular detection and analysis of green seaweeds from Rudong coasts in Jiangsu Province (in Chinese). *Chin Sci Bull (Chin Ver)*, 2011, 56: 309–317
- 20 Wang J F, Li N, Jiang P, et al. *Ulva* and *Enteromorpha* (Ulvaceae, Chlorophyta) from two sides of the Yellow Sea: Analysis of nuclear rDNA ITS and plastid *rbcL* sequence data. *Chin J Oceanol Limnol*, 2010, 28: 762–768
- 21 Pang S J, Liu F, Shan T F, et al. Tracking the algal origin of the *Ulva* bloom in the Yellow Sea by a combination of molecular, morphological and physiological analyses. *Mar Environ Res*, 2010, 69: 207–215
- 22 Zhang X W, Wang H X, Mao Y Z, et al. Somatic cells serve as a potential propagule bank of *Enteromorpha prolifera* forming a green tide in the Yellow Sea, China. *J Appl Phycol*, 2010, 22: 173–180
- 23 Shen S D, Li Y, Wu X, et al. Sequences and phylogeny analysis of *rbcL* gene in marine Chlorophyta. *J Ocean Univ China*, 2010, 9: 145–150
- 24 Liu H Y. Genetic diversity of *Enteromorpha* along the China coast (in Chinese). Doctoral dissertation. Beijing: Graduate University of Chinese Academy of Sciences, 2011
- 25 Leliaert F, Zhang X W, Ye N H, et al. Identity of the Qingdao algal bloom. *Phycol Res*, 2009, 57: 147–151
- 26 Wang W J, Wang F J, Cen S L, et al. PCR amplification and se-

- quence analysis of ITS regions of *Enteromorpha prolifera* (in Chinese). Mar Fish Res, 2008, 29: 124–129
- 27 Liang C W, Zhang X W, Su F, et al. Analysis of diversity of *Ulva* in Qingdao after the largest macroalgal bloom (in Chinese). Mar Sci Bull, 2010, 29: 540–545
  - 28 Mu X W, Lu Q Q, Hu C M, et al. Main species and seasonal changes of large green algae in the Jiangsu coastal area (in Chinese). J Nanjing Univ, 2011, 47: 470–480
  - 29 Ying C Q, Zhang T, Li X S, et al. Analysis of the similarity on ITS and 18S rDNA sequence of the main species from free-floating *Enteromorpha* along Chinese coast (in Chinese). J Fish Sci China, 2009, 33: 215–219
  - 30 Keesing J K, Liu D Y, Fearn P, et al. Inter- and intra-annual patterns of *Ulva prolifera* green tides in the Yellow Sea during 2007–2009, their origin and relationship to the expansion of coastal seaweed aquaculture in China. Mar Pollut Bull, 2011, 62: 1169–1182
  - 31 Zhao J, Jiang P, Li N, et al. Analysis of genetic variation within and among *Ulva pertusa* (Ulvacae, Chlorophyta) populations using ISSR markers. Chin Sci Bull, 2010, 55: 705–711
  - 32 Leskinen E, Pamilo P. Evolution of the ITS sequences of ribosomal DNA in *Enteromorpha* (Chlorophyceae). Hereditas, 1997, 126: 17–23
  - 33 Larkin M A, Blackshields G, Brown N P, et al. Clustal W and Clustal X version 2.0. Bioinformatics, 2007, 23: 2947–2948
  - 34 Tamura K, Dudley J, Nei M, et al. MEGA4: Molecular evolutionary genetics analysis (MEGA) software version 4.0. Mol Biol Evol, 2007, 24: 1596–1599
  - 35 Gao S, Chen X, Yi Q, et al. A strategy for the proliferation of *Ulva prolifera*, main causative species of green tides, with formation of sporangia by fragmentation. PLoS One, 2010, 5: e8571
  - 36 Gao S, Shen S D, Wang G C, et al. PSI-driven cyclic electron flow allows intertidal macro-algae *Ulva* sp. (Chlorophyta) to survive in desiccated conditions. Plant Cell Physiol, 2011, 52: 885–893
  - 37 Luo M B, Liu F. Salinity-induced oxidative stress and regulation of antioxidant defense system in the marine macroalga *Ulva prolifera*. J Exp Mar Biol Ecol, 2011, 409: 223–228
  - 38 Niu J F, Hu H Y, Hu S N, et al. Analysis of expressed sequence tags from the *Ulva prolifera* (Chlorophyta). Chin J Oceanol Limnol, 2010, 28: 26–36
  - 39 Jia S, Wang X, Liu G, et al. Gene expression analysis of “green tide” alga *Ulva prolifera* (Chlorophyta) in China. Genes Genom, 2011, 33: 173–178
  - 40 Lin H Z, Jiang P, Zhang J X, et al. Genetic and marine cyclonic eddy analyses on the largest macroalgal bloom in the world. Environ Sci Technol, 2011, 45: 5996–6002
  - 41 Zhao J, Jiang P, Liu Z Y, et al. Genetic variation of *Ulva* (*Enteromorpha*) *prolifera* (Ulvaes, Chlorophyta)—The causative species of the green tides in the Yellow Sea, China. J Appl Phycol, 2011, 23: 227–233

**Open Access** This article is distributed under the terms of the Creative Commons Attribution License which permits any use, distribution, and reproduction in any medium, provided the original author(s) and source are credited.